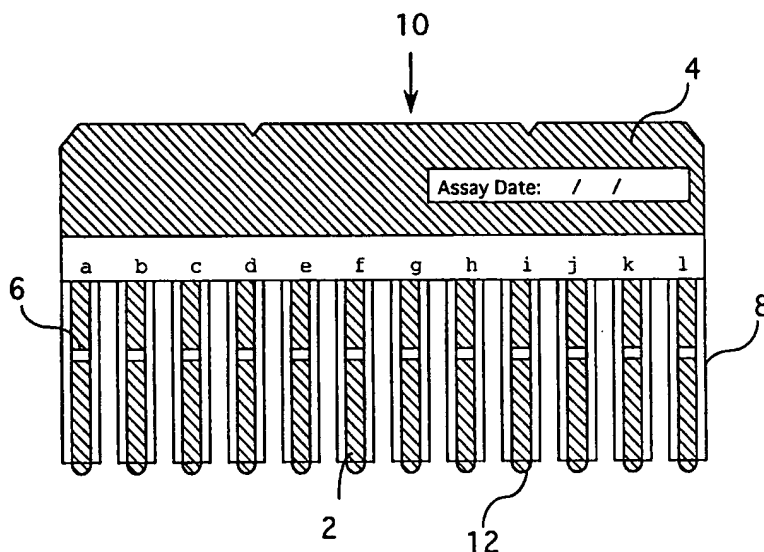




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US93/08759 <b>(22) International Filing Date:</b> 16 September 1993 (16.09.93)  <b>(30) Priority data:</b> 947,216                      18 September 1992 (18.09.92) US  <b>(71) Applicant:</b> ABBOTT LABORATORIES [US/US]; Chad 0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US).  <b>(72) Inventors:</b> TASKAR, Suhas, P. ; 38462 Burr Oak Lane, Wadsworth, IL 60083 (US). MALIEKAL, George ; 1298 Thorndale Lane, Lake Zurich, IL 60047 (US). SPRONK, Adrian, M. ; 2115 Witchwood Lane, Lindenhurst, IL 60046 (US).		<b>(74) Agents:</b> GORMAN, Edward, H., Jr. et al.; Abbott Laboratories, CHAD-0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US).  <b>(81) Designated States:</b> AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>

**(54) Title:** MULTIPLE ASSAY TEST STRIP DEVICES**(57) Abstract**

The present invention provides an assay device (10) having a plurality of strips (2) affixed to one another by a handling means (4) which may (i) either contact the distal ends of the strips (2), wherein the strips (2) are separated by a distance suitable to allow the strips (2) to be simultaneously contacted to individual test samples; or, (ii) may involve a card upon which each of the strips (2) is individually mounted. The test samples travel from the proximal end (12) to the distal end of each strip (2) by capillary action, and the strips contain an immobilized capture reagent (6) which binds target analyte, an ancillary specific binding member or a labeled reagent, in proportion to the amount or presence of target analyte in the test sample. The handling means (4) simplifies the assay procedure and decreases the amount of time needed to perform a large number of assays. Preferably, a procedural control is provided on each strip (2).

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## MULTIPLE ASSAY TESTSTRIP DEVICES

## BACKGROUND OF THE INVENTION

5 1. Field of the Invention

The present invention relates to a novel test device for detecting an analyte in a test sample by means of a binding assay. In particular, this invention relates to a novel test device for the simultaneous assay of multiple test samples.

10 2. Description of Related Art

The ability to use materials which specifically bind to an analyte of interest has created a need for diagnostic devices based on the use of binding assays. Binding assays incorporate specific binding members, typified by antibody and antigen immunoreactants, wherein one member of the specific binding pair is labeled with a  
15 signal-producing compound (e.g., an antibody labeled with an enzyme, a fluorescent compound, a chemiluminescent compound, a radioactive isotope, a direct visual label, etc.). For example, in a binding assay the test sample suspected of containing analyte can be mixed with a labeled reagent, e.g., labeled anti-analyte antibody, and incubated for a period of time sufficient for the immunoreaction to occur. The reaction mixture  
20 is subsequently analyzed to detect either that label which is associated with an analyte/labeled reagent complex (bound labeled reagent) or that label which is not complexed with analyte (free labeled reagent). As a result, the amount of free or bound label can be correlated to the amount of analyte in the test sample.

The solid phase assay format is a commonly used binding assay technique. There  
25 are a number of assay devices and procedures wherein the presence of an analyte is indicated by the analyte's capacity to bind to a labeled reagent and an immobilized or insoluble complementary binding member. The immobilized binding member is bound, or becomes bound during the assay, to a solid phase such as a dipstick, teststrip, flow-through pad, paper, fiber matrix or other suitable solid phase material. The binding  
30 reaction between the analyte and the assay reagents results in a distribution of the labeled reagent between that which is immobilized upon the solid phase and that which remains free. The presence or amount of analyte in a test sample is typically indicated by the extent to which the labeled reagent becomes immobilized upon the solid phase material.

35 The use of reagent-impregnated teststrips in specific binding assays is well-known. In such procedures, a test sample is applied to one portion of the teststrip and is allowed to migrate or wick through the strip material. Thus, the analyte to be detected or measured passes through or along the material, possibly with the aid of an

eluting solvent which can be the test sample itself or a separately added solution. The analyte migrates into a capture or detection zone on the teststrip, wherein a complementary binding member to the analyte is immobilized. The extent to which the analyte becomes bound in the detection zone can be determined with the aid of the  
5 labeled reagent which can also be incorporated in the teststrip or which can be applied separately.

In general, teststrips involve a material capable of transporting a solution by capillary action, i.e., a wicking or chromatographic action. Different areas or zones in the teststrip contain the assay reagents needed to produce a detectable signal as the  
10 analyte is transported to or through such zones. The device is suited for both chemical assays and binding assays and uses a developer solution to transport analyte along the strip.

The disadvantages of the conventional teststrip devices is that a single device was needed for each patient sample. The handling of multiple devices complicates the  
15 assay procedure and increases the amount of time needed to perform a large number of assays.

## SUMMARY OF THE INVENTION

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The present invention provides assay devices for determining the presence or amount of an analyte in a test sample containing or suspected of containing an analyte of interest. In one embodiment, the assay device involves a plurality of strips affixed to one another by a handling means wherein the handling means contacts the distal ends of  
25 the strips thereby forming a comb-shaped device. The strips are separated by a distance suitable to allow the strips to be simultaneously contacted to individual test samples. The test samples can travel from the proximal end to about the distal end of each strip by capillary action. The strips contain an immobilized capture reagent which is selected to bind to either the analyte, an ancillary specific binding member or  
30 a labeled reagent, in proportion to the amount or presence of analyte in the test sample.

Typically, the labeled reagent is contained within the strips in a situs between the proximal end and the immobilized capture reagent. The labeled reagent is selected to bind to either the analyte, an ancillary specific binding member or the immobilized capture reagent, thereby forming a detectable complex. Preferably, the labeled reagent  
35 is contained within the strips, and most preferably, the label is directly visually detectable without need for any further signal producing reaction, thereby making the assay essentially self-performing. In an alternative embodiment, the device further includes a plurality of application pads, each pad in fluid flow contact with a proximal

end of a strip, wherein the application pads contain a labeled reagent capable of migrating from the application pads to the strips upon the application of test sample, and wherein the labeled reagent binds to the analyte, an ancillary specific binding member or the capture reagent.

5       The device may be further modified wherein the handling means contains an identification means for each of the test samples, and/or instructions for performing the assay. Preferably, the device further contains a control reagent which is capable of reacting with a binding member immobilized in a control zone downstream from the immobilized capture reagent.

10       The device may alternatively involve a plurality of strips affixed to a handling means thereby forming a card-shaped device, wherein the strips are separated by a distance suitable to allow the strips to be simultaneously contacted to individual test samples. Each strip has a proximal end and a distal end, wherein the test sample can travel from the proximal end to about the distal end by capillary action. Each strip  
15       contains a zone of immobilized capture reagent which is selected to bind to either the analyte, an ancillary specific binding member or a labeled reagent. Application pads are in fluid flow contact with the proximal end of the strips, wherein the application pads contain a labeled reagent capable of migrating from the application pads to the strips wherein the reagent binds to the analyte, the ancillary specific binding member  
20       or the capture reagent. Such a device may also include a control reagent.

The test kits of the present invention contain the analytical device and may include additional instructions and/or ancillary reagents such as diluents and buffers.

## 25                   BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a frontal view of a comb-shaped device of the present invention.

Figure 2 is a frontal view of a card-shaped device of the present invention.

30

## DETAILED DESCRIPTION OF THE INVENTION

35       This invention provides assay devices and methods, where the devices use multiple strips of chromatographic material capable of transporting liquids for the simultaneous performance of an assay on multiple patient samples or the performance of a multiple assay on a single patient sample. The device may include test sample application pads, contacting the strips, which function to regulate the flow of test

sample to the chromatographic material, to filter the test samples and to deliver and/or mix assay reagents. Assay reagents may be incorporated within the application pad as well as the chromatographic material. By varying the configuration of reagent-containing sites on the device, qualitative and quantitative displays of assay results can be obtained. The devices can be formatted to perform binding assays, such as the complementary binding of an antigen and antibody. The detectable signal resulting from the binding assay can then be detected by instrumentation or direct visual observation.

The present invention is particularly advantageous in that it combines several elements to form a novel assay device with which a one-step assay can be performed for a number of different patient samples at one time. The novel device simplifies the assay protocols by decreasing the number of manual steps required for its use, thereby reducing the risk of errors during use. The combination of elements in the present invention also enables the use of predetermined amounts of reagents incorporated within the device, thereby avoiding the need for reagent measurements and additions by the user. Furthermore, the reagents are situated in the device in such a way as to make the assay substantially self-performing and to facilitate the detection and quantitation of the assay results.

#### I. DEFINITIONS

Before proceeding with the description of the various embodiments of the present invention, a number of terms used herein will be defined.

"Test sample" refers to a material suspected of containing the analyte. The test sample can be used directly as obtained from the source or after pretreatment so as to modify its character. The test sample can be derived from any source, such as a physiological fluid, including, blood, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, amniotic fluid or the like. The test sample can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, or the like; methods of treatment can involve filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples can be used such as water, food products and the like for the performance of environmental or food production assays as well as diagnostic assays. In addition, a solid material suspected of containing the analyte can be used as the test sample once it is modified to form a liquid medium or to release the analyte.

"Specific binding member" refers to a member of a specific binding pair, i.e., two different molecules wherein one of the molecules specifically binds to the second molecule through chemical or physical means. In addition to antigen and antibody specific binding pair members, other specific binding pairs include, as examples

without limitation, biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, polymeric acids and bases, dyes and protein binders, peptides and specific protein binders (e.g.,  
5 ribonuclease, S-peptide and ribonuclease S-protein), and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding member, for example an analyte-analog or a specific binding member made by recombinant techniques or molecular engineering. If the specific binding member is an  
10 immunoreactant it can be, for example, an antibody, antigen, hapten, or complex thereof, and if an antibody is used, it can be a monoclonal or polyclonal antibody, a recombinant protein or antibody, a chimeric antibody, a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific  
15 binding members are well-known to those skilled-in-the-art.

"Analyte" or "analyte of interest" refers to the compound or composition to be detected or measured, which has at least one epitope or binding site. The analyte can be any substance for which there exists a naturally occurring analyte-specific binding member or for which an analyte-specific binding member can be prepared. Analytes  
20 include, but are not limited to toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), and metabolites of or antibodies to any of the above substances. The term "analyte" also includes any antigenic substances, haptens, antibodies,  
25 macromolecules and combinations thereof.

"Analyte-analog" refers to a substance which cross-reacts with the analyte-specific binding member, although it may do so to a greater or a lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a fragmented or synthetic portion of the analyte molecule, so long as the analyte-analog  
30 has at least one epitopic site in common with the analyte of interest. An example of an analyte-analog is a synthetic peptide sequence which duplicates at least one epitope of the whole-molecule analyte so that the analyte-analog can bind to the analyte-specific binding member.

"Labeled reagent" refers to a substance comprising a detectable label attached to  
35 a specific binding member. The attachment may be covalent or non-covalent binding, but the method of attachment is not critical to the present invention. The label allows the labeled reagent to produce a detectable signal that is directly or indirectly related to the amount of analyte in the test sample. The specific binding member component of the

labeled reagent is selected to directly bind to the analyte or to indirectly bind the analyte by means of an ancillary specific binding member, which is described in greater detail hereinafter. The labeled reagent can be incorporated into the test device, it can be combined with the test sample to form a test solution, it can be added to the device separately from the test sample or it can be predeposited or reversibly immobilized at the capture site. In addition, the binding member may be labeled before or during the performance of the assay by means of a suitable attachment method.

"Label" refers to any substance which is capable of producing a signal that is detectable by visual or instrumental means. Various labels suitable for use in the present invention include labels which produce signals through either chemical or physical means. Such labels can include enzymes and substrates; chromogens; catalysts; fluorescent compounds; chemiluminescent compounds; radioactive labels; direct visual labels including colloidal metallic particles such as gold, colloidal non-metallic particles such as selenium, dyed or colored particles such as a dyed plastic or a stained microorganism, colored or colorable organic polymer latex particles, and liposomes or other vesicles containing directly visible substances; and the like.

The selection of a particular label is not critical to the present invention, but the label will be capable of generating a detectable signal either by itself, such as a visually detectable colored organic polymer latex particle, or instrumentally detectable, such as a fluorescent compound, or detectable in conjunction with one or more additional signal producing components, such as an enzyme/substrate signal producing system. A variety of different labeled reagents can be formed by varying either the label or the specific binding member component of the labeled reagent; it will be appreciated by one skilled-in-the-art that the choice involves consideration of the analyte to be detected and the desired means of detection.

"Signal producing component" refers to any substance capable of reacting with another assay reagent or with the analyte to produce a reaction product or signal that indicates the presence of the analyte and that is detectable by visual or instrumental means. "Signal production system", as used herein, refers to the group of assay reagents that are needed to produce the desired reaction product or signal. For example, one or more signal producing components can be reacted with the label to generate a detectable signal, e.g., when the label is an enzyme, amplification of the detectable signal is obtained by reacting the enzyme with one or more substrates or additional enzymes and substrates to produce a detectable reaction product.

In a preferred embodiment of the present invention, a visually detectable label is used as the label component of the labeled reagent, thereby providing for the direct visual or instrumental readout of the presence or amount of the analyte in the test sample without the need for additional signal producing components at the detection



sites. Suitable materials for use include colloidal metals, such as gold, and dye particles. Non-metallic colloids, such as colloidal selenium, tellurium and sulfur particles may also be used.

"Immobilized reagent" refers to a specific binding member that is attached  
5 within or upon a portion of the solid phase support or chromatographic strip to form a "capture site" wherein the analyte and/or labeled reagent become immobilized on the strip or wherein the immobilized reagent slows the migration of the analyte and/or labeled reagent through the strip. The method of attachment is not critical to the present invention. The capture reagent facilitates the observation of the detectable  
10 signal by substantially separating the analyte and/or the labeled reagent from unbound assay reagents and the remaining components of the test sample. Typically, the immobilized reagent is selected to bind the analyte, the labeled reagent or a complex thereof. In preferred embodiments, the immobilized reagent binds to the analyte for the completion of a sandwich complex. The immobilized reagent may be chosen to  
15 directly bind the analyte or indirectly bind the analyte by means of an ancillary specific binding member which is bound to the analyte. In addition, the immobilized reagent may be immobilized on the solid phase before or during the performance of the assay by means of any suitable attachment method.

Typically, the capture site of the present invention is a delimited or defined  
20 portion of the solid phase support such that the specific binding reaction between the immobilized reagent and analyte is localized or concentrated in a delimited site. This facilitates the detection of label that is immobilized at the capture site in contrast to other portions of the solid phase support. The delimited site is typically less than 50% of the solid phase support, and preferably less than 10% of the solid phase support.  
25 The immobilized reagent can be applied to the solid phase material by dipping, inscribing with a pen, dispensing through a capillary tube or through the use of reagent jet-printing or any other suitable dispensing techniques. In addition, the capture site can be marked, for example with a dye, such that the position of the capture site upon the solid phase material can be visually or instrumentally determined even when there  
30 is no label immobilized at the site. Preferably, the immobilized reagent is positioned on the strip such that the capture site is not directly contacted with the test sample, that is, the test sample must migrate by capillary action through at least a portion of the strip before contacting the immobilized reagent.

The immobilized reagent may be provided in a single capture or detection site or  
35 in multiple sites on or in the solid phase material. The immobilized reagent may also be provided in a variety of configurations to produce different detection or measurement formats. Alternatively, the immobilized reagent can be distributed over a large portion of the solid phase material in a substantially uniform manner to form

the capture site. The extent of signal production in the capture site is related to the amount of analyte in the test sample.

"Ancillary specific binding member" refers to any member of a specific binding pair which is used in the assay in addition to the specific binding members of the labeled reagent or immobilized reagent. One or more ancillary specific binding members can be used in an assay. For example, an ancillary specific binding member can be capable of binding the labeled reagent to the analyte of interest, in instances where the analyte itself could not directly attach to the labeled reagent. Alternatively, an ancillary specific binding member can be capable of binding the immobilized reagent to the analyte of interest, in instances where the analyte itself could not directly attach to the immobilized reagent. The ancillary specific binding member can be incorporated into the assay device or it can be added to the device as a separate reagent solution.

## II. DEVICE COMPONENTS

### a. Strip

"Solid phase support" or "chromatographic material" or "strip" refers to any suitable porous, absorbent, bibulous, isotropic or capillary material, which includes the reaction site of the device and through which the analyte or test solution can be transported by a capillary or wicking action. It will be appreciated by one skilled-in-the-art that the strip can be made of a single material or more than one material (e.g., different zones, portions, layers, areas or sites can be made of different materials) so long as the multiple materials are in fluid-flow contact with one another thereby enabling the passage of test sample between the materials. Fluid-flow contact permits the passage of at least some components of the test sample, e.g., analyte, between the zones of the porous material and is preferably uniform along the contact interface between the different zones.

Natural, synthetic, or naturally occurring materials that are synthetically modified, can be used as the solid-phase support and include, but are not limited to: papers (fibrous) or membranes (microporous) of cellulose materials such as paper, cellulose, and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels; and the like. The porous material should not interfere with the production of a detectable signal. The chromatographic material may have an inherent strength, or strength can be provided by means of a supplemental support.

35

### b. Application pad

An optional device component is a test sample application pad. The application pad is in fluid flow contact with one end of the strip material, referred to as the

proximal end, such that the test sample can pass or migrate from the application pad to the strip. Fluid flow contact can include physical contact of the application pad to the chromatographic material as well as the separation of the pad from the strip by an intervening space or additional material which still allows fluid to pass between the pad and the strip. Substantially all of the application pad can overlap the chromatographic material to enable the test sample to pass through substantially any part of the application pad to the proximal end of the strip. Alternatively, only a portion of the application pad might be in fluid flow contact with the chromatographic material. The application pad can be any material which can transfer the test sample to the chromatographic material and which can absorb a volume of test sample that is equal to or greater than the total volume capacity of the chromatographic material.

Materials preferred for use in the application pad include nitrocellulose, porous polyethylene frit or pads and glass fiber filter paper. The material must also be chosen for its compatibility with the analyte and assay reagents, for example, glass fiber filter paper was found to be the preferred application pad material for use in a human chorionic gonadotropin (hCG) assay device.

In addition, the application pad typically contains one or more assay reagents either diffusively or non-diffusively attached thereto. Reagents which can be contained in the application pad include, but are not limited to, labeled reagents, ancillary specific binding members, and signal producing system components needed to produce a detectable signal. For example, in a binding assay it is preferred that the labeled reagent be contained in the application pad. The labeled reagent is released from the pad to the strip with the application of the test sample, thereby eliminating the need to combine the test sample and labeled reagent prior to using the device. The isolation of assay reagents in the application pad also keeps interactive reagents separate and facilitates the manufacturing process.

In some instances, the application pad also serves the function of an initial mixing site and a reaction site for the test sample and reagent. In preferred embodiments, the application pad material is selected to absorb the test sample at a rate that is faster than that achieved by the strip material alone. Typically, the pad material is selected to absorb fluids 2 to 5 time faster than the strip material. Preferably, the pad will absorb fluids 4 to 5 time faster than will the strip material.

In an optional embodiment of the present invention, gelatin is used to encompass all or part of the application pad. Typically, such encapsulation is produced by overcoating the application pad with fish gelatin. The effect of this overcoating is to increase the stability of the reagent contained by the application pad. The application of test sample to the overcoated application pad causes the gelatin to dissolve and thereby enables the dissolution of the reagent. In an alternative embodiment of the present

invention, the reagent containing application pad is dried or lyophilized to increase the shelf-life of the device. Lyophilized application pads were found to produce stronger signals than air dried application pads, and the lyophilized application pads maintained stability for longer periods. The reagents contained in the application pad are

5 rehydrated with the addition of test sample to the pad.

The present invention can be further modified by the addition of a filtration means. The filtration means can be a separate material placed above the application pad or between the application pad and the strip material, or the material of the application pad itself can be chosen for its filtration capabilities. The filtration means can include

10 any filter or trapping device used to remove particles above a certain size from the test sample. For example, the filter means can be used to remove red blood cells from a sample of whole blood, such that plasma is the fluid received by the application pad and transferred to the chromatographic material.

Yet another modification of the present invention involves the use of an

15 additional layer or layers of porous material placed between the application pad and the chromatographic material or overlaying the application pad. Such an additional pad or layer can serve as a means to control the rate of flow of the test sample from the application pad to the strip. Such flow regulation is preferred when an extended incubation period is desired for the reaction of the test sample and the reagent(s) in the

20 application pad. Alternatively, such a layer can contain an additional assay reagent(s) which is preferably isolated from the application pad reagents until the test sample is added, or it can serve to prevent unreacted assay reagents from passing to the chromatographic material.

When small quantities of non-aqueous or viscous test samples are applied to the

25 application pad, it may be necessary to employ a wicking or transport solution, preferably a buffered solution, to carry the reagent(s) and test sample from the application pad and through the strip. When an aqueous test sample is used, a transport solution generally is not necessary but can be used to improve flow characteristics through the device or to adjust the pH of the test sample. The transport solution

30 typically has a pH range from about 5.5 to about 10.5, and more preferably from about 6.5 to about 9.5. The pH is selected to maintain a significant level of binding affinity between the specific binding members in a binding assay. When the label component of the indicator reagent is an enzyme, however, the pH also must be selected to maintain significant enzyme activity for color development in enzymatic signal production

35 systems. Illustrative buffers include phosphate, carbonate, barbital, diethylamine, tris, 2-amino-2-methyl-1-propanol and the like. The transport solution and the test sample can be combined prior to contacting the application pad or they can be contacted to the application pad sequentially.

Predetermined amounts of signal producing components and ancillary reagents can be incorporated within the device, thereby avoiding the need for additional protocol steps or reagent additions. Thus, it is also within the scope of this invention to provide more than one reagent to be immobilized within the application pad and/or the strip material.

The particular dimensions of the strip material will be a matter of convenience, depending upon the size of the test sample involved, the assay protocol, the means for detecting and measuring the signal, and the like. For example, the dimensions may be chosen to regulate the rate of fluid migration as well as the amount of test sample to be imbibed by the chromatographic material.

It is necessary to select strip dimensions which allow the combination of multiple strips in a single assay device. In a "comb-type" device, multiple strips are attached by a handling means at the end distal from the test sample application portion. Such a device is depicted in Figure 1. The test sample application portion of each of the strips is separated by a distance suitable to allow the strips to be simultaneously contacted to individual test samples. In one embodiment, the strips are separated on the comb-type device such that each of the strips can be simultaneously contacted to an individual test sample container such as a microtiter well. For example, the comb-type device may involve as many as ten or twelve connected strips which can be simultaneously contacted to an equal number of individual test samples.

In an alternative embodiment, multiple strips may be carried on a single "card-type" device, as depicted in Figure 2. Such a device is suitable for the one-step performance of a selected assay on a number of different patient samples, after which the entire device can be readily disposed. The dimensions of the handling means on which the strips are mounted is selected for ease of handling and disposal as well as to contain strips having dimensions suitable for the assay of interest.

The strips may be affixed to the handling means by an adhesive or a laminate layer may connect the strips and the handling means. It is also within the scope of the present invention for the handling means to be an extension of the material that forms the strips. In a preferred embodiment, the handling means of the card-type and comb-type devices are constructed such that the device may be cut or disconnected so that the number of strips matches the number of samples to be tested.

The comb-type and card-type devices may involve only the strip material with the addition of a labeled reagent simultaneously or sequentially with the test sample. Alternatively, the individual strips may be in contact with individual application pads which contain the labeled reagent. Reagents can be added directly to either the application pad or the chromatographic material during the performance of the assay. The preferred embodiment of the invention, however, involves the incorporation of all

necessary assay reagents into the assay device so that the test samples need only be contacted to the application pads to perform the assays. Therefore, one or more assay reagents can be present in either or both the application pad or chromatographic material of the present invention. Preferably, the handling means will include the  
5 instructions for performing the assay as well as a means for identifying the individual test samples.

It is also within the scope of this invention to have a reagent, at the distal end of the chromatographic material, which indicates the completion of a binding assay (i.e., end of assay indicator) by changing color upon contact with the test solution, wicking  
10 solution or a signal producing component. Reagents which would change color upon contact with a test solution containing water are the dehydrated transition metal salts, such as  $\text{CuSO}_4$ ,  $\text{Co}(\text{NO}_3)_2$ , and the like. The pH indicator dyes can also be selected to respond to the pH of the buffered wicking solution. For example, phenolphthalein changes from clear to intense pink upon contact with a wicking solution having a pH  
15 range between 8.0-10.0.

In a preferred embodiment a procedural control is provided on each of the strips. The binding of the control reagent to the control site or bar demonstrates that the assay reagents were reactive and that the assay reactions took place as planned. The proportion of control reagent to labeled reagent is adjusted such that the appearance of  
20 the procedural control reaction occurs at the same time or later than the appearance of the assay result at the capture zone.

The control reagent can involve a labeled binding member that is related or unrelated to the analyte of interest. In addition, the label used in the control reagent may be identical to or different than the label used in the labeled reagent. For example,  
25 the control reagent may be labeled anti-horse globulin antibody which is unrelated to the analyte and does not react with the analyte or the immobilized capture reagent. The control bar then contains a binding member, specific for the control reagent, e.g., horse globulin, immobilized on the strip downstream from the capture site. The control reagent may be combined with the labeled reagent such that both pass through  
30 the strip simultaneously. The control reagent passes through the capture zone to the control bar where it is immobilized and detected. Alternatively, the labeled reagent itself may serve as the control reagent. For example, labeled antibody to hepatitis B surface antigen forms a sandwich complex with the HBsAg analyte and immobilized anti-HBsAg antibody at the capture zone, while the control bar contains recombinant  
35 HBsAg to capture that unbound labeled reagent which passes through the capture zone. In yet another embodiment, the binding member component of the control reagent and the analyte-binding member of the labeled reagent may be conjugated to a single label such a colloidal dye particle or a colloidal selenium particle.

When using a visually detectable particles as the label, such as selenium, dyed particles or black latex, the labeled reagent binding member and the control reagent binding member may both be attached to the particles. Alternatively, the binding members may be attached to separate batches of particles and the particles are then mixed.

The present invention further provides kits for carrying out binding assays. For example, a kit according to the present invention can comprise the comb-type or card-type device with its incorporated reagents as well as a transport solution and/or test sample pretreatment reagent as described above. Other assay components known to those skilled in the art, include buffers, stabilizers, detergents, bacteria inhibiting agents and the like which can also be present in the assay device or separate reagent solution.

The present invention optionally includes a nonreactive cover or enclosure around the device. Preferably, the cover encloses at least the strip to avoid contact with and contamination of the capture site. The cover may also include a raised area adjacent to the application pad to facilitate receiving and/or containing a certain volume of the test sample and/or wicking solution. It is preferred that a sufficient portion of the strip be encased to prevent applied test sample from contacting the capture site without first passing through a portion of the strip.

In Figure 1, an assay device (10) is depicted with the individual strips (2) connected by a handling means (4), as described above. The capture situs (6) represents an embodiment, as described above, wherein immobilized capture reagent is distributed as a bar which traverses the width of the chromatographic material. The strips are enclosed by a cover means (8) such that at least a portion of the proximal end (12) of the strip may be contacted to a test sample.

In Figure 2, an assay device (10) is depicted with the individual strips (2) connected by a card-shaped handling means (4), as described above. The application pads (14) are located at the proximal end of the strips. The capture situs (6) is an immobilized capture reagent distributed as a bar which traverses the width of the chromatographic material. In a preferred embodiment, the strips are enclosed by a cover means such that only a portion of the application pads may be contacted to a test sample.

The following examples are given by way of illustration only and should not be construed as limiting the scope of the invention as based upon this disclosure. Many variations on the present invention will become obvious to those of ordinary skill in the art.

## EXAMPLES

## Example 1

## Assay for Hepatitis B Surface Antigen (HBsAg)

5           A comb-type device was constructed with 12 strips per comb. The strips were formed from nitrocellulose with dimensions selected such that the strips could be simultaneously inserted into a row of wells in a microtiter plate. A capture site was formed on each strip by immobilizing a monoclonal anti-HBsAg antibody (4-5 mg/ml) as a bar which traversed the width of each strip and was positioned in about the center of the length of each strip.

10           The individual test samples (50  $\mu$ l) were placed in the wells with a labeled reagent (15  $\mu$ l) containing visibly detectable particles of black latex coated with a polyclonal anti-HBsAg antibody. The comb-type device was then inserted into the wells so that the strip tips contacted the bottom of the wells. The reaction mixture was

15           allowed to migrate through the strips for 15 minutes.

          A dark line appeared at the capture site of any strip contacted to a test sample containing HBsAg. In this experiment, the detection limit of the assay was 1-3 nanograms of HBsAg per milliliter of test sample.

20

## Example 2

## Assay for Hepatitis B Surface Antigen (HBsAg)

          The device was constructed substantially in accordance with description of Example 1 with the addition of a procedural control. The control involved the

25           immobilization of an anti-label antibody at a point on the strip downstream from the capture site. The sandwich assay was performed as described in Example 1, and the results are depicted in Table 1.

Table 1

Time required for visual signal development  
at the capture site  
(minutes)

Concentration of HBsAg/ml (ng)

50

2

10

5

5

7

2

12

1

15

0

no visual signal

Procedural control

2 minutes



The test result was valid if the procedural bar was visible at the time of reading. The test sample was negative if only the procedural bar was visible. The test sample was positive if the procedural bar and the capture bar were visible at the time of reading.

5

### Example 3

#### One Step Immunochromatographic Assay For Benzoyllecgonine (Cocaine Metabolite)

10 a. Indicator reagent preparation - selenium colloid/anti-benzoyllecgonine antibody conjugate

A 3% stock solution of  $\text{SeO}_2$  (50 ml, 3 g/ml, Aldrich) is diluted in water (5 L) and the pH is adjusted first with 2% NaOH to 4.4-4.5 and then with 2%  $\text{K}_2\text{CO}_3$  to 4.95-5.05. The solution is heated to 75-80 °C. The solution is mixed with a 5% ascorbate solution (75 ml), and this reaction mixture is heated in a 70 °C water bath while stirring for five minutes. The solution is then mixed with 5% ascorbate (6 ml/L), and this reaction mixture is heated in a 70 °C water bath while stirring for ten minutes. The solution is then allowed to cool to room temperature.

20 Approximately 10 µg of affinity purified rabbit anti-benzoyllecgonine antibody (Berkeley Antibody Company, Inc., Richmond, CA) dissolved in phosphate-buffered saline (PBS, pH 7.4) is added to the selenium suspension (5 ml). The mixture is gently mixed for about ten minutes. A 5% BSA solution (bovine serum albumin, 300 µl) is added to the mixture, and the mixture is gently mixed and then is allowed to stand for about ten minutes. Five milliliters of the selenium colloid/anti-benzoyllecgonine antibody indicator reagent (1.89 µg/ml) is added to five milliliters of conjugate diluent (0.1M Tris, 4% sucrose, 2% surfactant [Pluronic-68, BASF Corporation, Parsippany, NJ] and 4% casein) for a final concentration of 0.97 µg/ml.

30 Conjugate diluent is prepared by adding Tris (1.2 g), sucrose (4.0 g) and Pluronic-68 (2.0 g) to distilled water (70 ml) at pH 8.1. Alkaline treated casein (4.0 g) is added to the solution, and the pH is adjusted to approximately 8.1. The volume is brought to 100 milliliters, and the mixture is filtered through a 0.2 µm filter.

b) Application pad preparation

35 Indicator reagent (30 µl, from Example 3.a) is applied to each application pad, and the pads may either be air dried at room temperature or lyophilized. Selected application pad materials are either porous polyethylene (4 mm x 10 mm x 1.5 mm rectangles of Porex material, Porex Technologies, Fairburn, GA) or glass fiber paper

(4 mm x 10 mm rectangles of resin bonded glass fiber paper, Lypore-9254, Lydall Incorporated, Rochester, NH).

c) Chromatographic material preparation

5        Capture sites are prepared by reagent printing four different reagents as parallel bars, about two millimeters wide and approximately one-quarter inch apart, using sheets of nitrocellulose (8 inch x 10 inch, 5.0  $\mu$ M, Schleicher and Schuell, Keene, NH). The most proximal bar is benzoylecgonine:BSA (17:1) at 1 mg/ml. The next bar is goat anti-rabbit antibody (affinity isolated, Sigma Chemical Company, St.  
10    Louis, MO) at 0.5 mg/ml. The next bar is goat anti-mouse antibody (affinity isolated, Sigma) at 0.5 mg/ml. The final bar is a blend of equal portions of both the goat anti-rabbit antibody and the goat anti-mouse antibody. The filter is then cut into 3 millimeters x 50 millimeters strips such that the cut was perpendicular to the printed bars. The application pads of Example 3.b are placed in fluid-flow contact with the  
15    proximal ends of the chromatographic strips.

d) Device preparation

20        The individual strips are affixed to a handling means using an adhesive which does not interfere with the reaction of the reagents or the transport of fluids through the pad or strip materials.

e) Assay protocol

25        Benzoylecgonine test samples (30  $\mu$ l of 0  $\mu$ g/ml or 5.0  $\mu$ g/ml sample concentrations) are contacted to the application pads. The test samples pass through the application pads wherein any benzoylecgonine in the test sample mixes with the reconstituted labeled reagent and migrates through the strip. The labeled reagent/analyte complex bypasses the benzoylecgonine capture site and is immobilized  
30    at the goat anti-rabbit antibody bar where the formation of the immunocomplex is detected by the appearance of a dark signal over substantially the entire bar. The test samples which do not contain benzoylecgonine reconstitute the labeled reagent, and that unbound reagent will be substantially captured at the benzoylecgonine capture site thereby producing a visible sharp thin line.

35

Example 4

One Step Immunochromatographic Assay For Hepatitis B Surface Antigen (HBsAg)

a) Labeled reagent preparation - selenium colloid/anti-biotin antibody conjugate

A selenium colloid suspension is prepared substantially in accordance with the procedure of Example 4. Two milliliters of the selenium suspension is mixed with 150 mM borate (44  $\mu$ l, pH 8.0). Rabbit anti-biotin antibody (6  $\mu$ l, 3  $\mu$ g/ml) is added, and  
5 the mixture is gently mixed for about ten minutes. Ten percent BSA (60  $\mu$ l) is added and mixed. The resulting conjugate is used without centrifugation. The selenium colloid/anti-biotin antibody conjugate (0.4 ml) is mixed with 5% casein for assay use.

10 b) Application pad preparation

Labeled reagent is applied to porous polyethylene pads (3/8 inch x 3/16 inch, Porex). The pads are dried at 40 °C for one hour.

c) Ancillary pad preparation

15 An ancillary specific binding member complex of biotin and anti-HBsAg antibody is prepared in accordance with procedures well-known in the art. The biotin/anti-HBsAg antibody complex (0.4 ml at 4  $\mu$ g/ml) is mixed with a diluent (0.4 ml, phosphate buffered saline with 1% BSA). This mixture is also applied to porous polyethylene pads (3/8 inch x 3/16 inch, Porex) which are dried at 40 °C for 1 hour.

20

d) Chromatographic material preparation

The chromatographic material is prepared by dotting anti-HBsAg antibody (0.3  $\mu$ l, 1.83 mg/ml) onto nitrocellulose (Schleicher and Schuell) to form the capture situs. The application pads are placed upon the proximal end of the chromatographic  
25 strips, and the ancillary pad is stacked upon the application pad.

e) Device preparation

The individual strips are affixed to a handling means using a cuttable adhesive which does not interfere with the reaction of the reagents or the transport of fluids  
30 through the pad or strip materials.

f) Assay protocol

HBsAg test samples (120  $\mu$ l of 0, 1.0, 2.5, 5.0, 10.0 and 5000 ng/ml sample concentrations) are contacted to the ancillary pads. The test samples pass through both  
35 the ancillary pads and the application pads thereby reconstituting both the biotin-anti-HBsAg antibody complex and the selenium colloid/anti-biotin antibody labeled reagent. If HBsAg is present in the test sample, then a selenium colloid/anti-biotin antibody/biotin-anti-HBsAg antibody/analyte complex is formed and migrates through

the strip. The complex is immobilized at the anti-HBsAg antibody capture situs where the formation of a "sandwich" immunocomplex is detected by the appearance of a dark signal.

5

## Example 5

## One Step Immunochromatographic Assay For hCG

a. Labeled reagent preparation - selenium colloid/anti-hCG antibody conjugate

A selenium colloid suspension is prepared. The selenium suspension (250 ml) is mixed with anti-hCG antibody (1250 µg), and the mixture is stirred for about 15 minutes. A BSA blocking solution (25 ml, comprising 5 gm of BSA and 5 ml of 1 M Tris at pH 7.4 with a sufficient quantity of water to bring the volume to 500 ml) is added to the mixture and stirred. The resulting selenium colloid/anti-hCG antibody conjugate is centrifuged (at 1900 x g) for 40 minutes at 4° C. The supernatant is removed, and BSA blocking solution (25 ml) is added to resuspend the pellet by vortexing. The centrifugation and aspiration process is repeated, and the final pellet is resuspended in BSA blocking solution (5 ml).

b) Application pad preparation

Indicator reagent (from Example 6.a) is diluted 1:30 with 145 milliliters of conjugate diluent comprising Tris (0.18 g, 10 mM at pH 7.4), 2% lactose (3.0 g) to decrease particle aggregation, alkaline treated casein (2%, 3.0 g) and goat IgG (300 mg, 2.0 mg/ml). The application pad material comprises resin bonded glass fiber paper (Lydall). The indicator reagent is coated onto the application pad material, the material is cut into 1/4 inch x 1/2 inch rectangles, and the pads are dried at 98° F.

c) Chromatographic material preparation

The capture situs is prepared by reagent printing anti-hCG antibody (0.1 mg/ml in TBS capture antibody diluent containing 0.1M Tris HCl at pH 7.4, 1.0% sucrose, and 0.1% BSA) onto nitrocellulose sheets (8 inch x 10 inch, 5.0 µM Schleicher and Schuell). The sheet is cut into strips such that the cut is perpendicular to the printed bar which contained approximately 0.25 µl of antibody per bar. The sheet is not completely cut so that the distal end of each strip is joined to the adjacent strip by a portion of the strip material. This handling means is further strengthened by the addition of a solid support. The application pads are placed over the bottom 3 millimeters of the strips, approximately 10 millimeters below the capture situs. The assay device was then enclosed in laminate layers of moisture impervious material leaving a portion of the application pads exposed.

The concepts of the present invention are applicable to various types of chemical and binding assays. It will be appreciated, however, that one skilled in the art can conceive of many other assays, including assays for analytes other than antigens or antibodies, to which the present inventive concepts can be applied. The embodiments  
5 described and the alternative embodiments presented are intended as examples rather than as limitations. Thus, the description of the invention is not intended to limit the invention to the particular embodiments disclosed, but it is intended to encompass all equivalents and subject matter within the scope of the invention as described above and as set forth in the following claims.

## CLAIMS

What is claimed is:

- 5 1. In an analytical device for determining the presence or amount of an analyte in a test sample, comprising a strip having a proximal end and a distal end, wherein the test sample can travel from said proximal end to about said distal end by capillary action, and wherein said strip contains an immobilized capture reagent which binds to a member selected from the group consisting of the analyte, an ancillary specific binding member and a labeled reagent,
- 10 the improvement comprising:  
a plurality of said strips affixed to one another by a handling means wherein said handling means contacts said distal ends of said strips thereby forming a comb-shaped device, and wherein said strips are separated by a distance suitable to allow the strips to be simultaneously contacted to individual test samples.
- 15 2. The device according to Claim 1, wherein a labeled reagent is contained within said strips in a situs between said proximal end and said immobilized capture reagent.
- 20 3. The device according to Claim 1, wherein said capture reagent is an immobilized anti-HBsAg antibody and said labeled reagent is a labeled anti-HBsAg antibody
4. The device according to Claim 1, further comprising: a plurality of application pads, each pad in fluid flow contact with a proximal end of a strip, wherein said application pads contain a labeled reagent capable of migrating from said application pads to said strips upon the application of test sample, and wherein said labeled reagent binds to a member selected from the group consisting of the analyte, said ancillary specific binding member and said capture reagent.
- 25 5. The device according to Claim 1, wherein said handling means contains an identification means for each of the test samples.
- 30 6. The device according to Claim 5, wherein said handling means further contains instructions for performing the assay.
- 35 7. The device according to Claim 1, further comprising a control reagent, wherein said control reagent is capable of reacting with a binding member immobilized in a control zone downstream from said immobilized capture reagent.

8. The device according to Claim 1, further comprising a labeled reagent and a binding member immobilized in a control zone downstream from said immobilized capture reagent, wherein said labeled reagent which passes through said immobilized capture reagent is immobilized at said control zone.

5

9. The device according to Claim 1, further comprising a control reagent, wherein said control reagent is capable of reacting with a binding member immobilized in a control zone downstream from said immobilized capture reagent and wherein said control reagent is labeled identically to said labeled reagent.

10

10. The device according to Claim 1, further comprising a control reagent, wherein said control reagent is capable of reacting with a binding member immobilized in a control zone downstream from said immobilized capture reagent, and wherein said control reagent is bound to the label component of said labeled reagent.

15

11. The device according to Claim 1, further comprising a cover means to enclose said strips and prevent the test sample from directly contacting said immobilized capture reagent.

20

12. The device according to Claim 1, wherein said handling means is an extension of said strips.

13. In an analytical device for determining the presence or amount of an analyte in a test sample, comprising a strip having a proximal end and a distal end, wherein the test sample can travel from said proximal end to about said distal end by capillary action, and wherein said strip contains an immobilized capture reagent which binds to a member selected from the group consisting of the analyte, an ancillary specific binding member and a labeled reagent,

25

the improvement comprising:

30

a plurality of said strips affixed to a handling means thereby forming a card-shaped device, and wherein said strips are separated by a distance suitable to allow the strips to be simultaneously contacted to individual test samples; and

35

application pads in fluid flow contact with said proximal end of said strips, wherein said application pads contain a labeled reagent capable of migrating from said application pads to said strips wherein said reagent binds to a member selected from the group consisting of the analyte, said ancillary specific binding member and said capture reagent.

14. The device according to Claim 13, further comprising a control reagent, wherein said control reagent is capable of reacting with a binding member immobilized in a control zone downstream from said immobilized capture reagent.
- 5 15. The device according to Claim 13, further comprising a labeled reagent and a binding member immobilized in a control zone downstream from said immobilized capture reagent, wherein said labeled reagent which passes through said immobilized capture reagent is immobilized at said control zone.
- 10 16. The device according to Claim 13, further comprising a control reagent, wherein said control reagent is capable of reacting with a binding member immobilized in a control zone downstream from said immobilized capture reagent, wherein said control reagent is labeled identically to said labeled reagent.
- 15 17. The device according to Claim 13, further comprising a control reagent, wherein said control reagent is capable of reacting with a binding member immobilized in a control zone downstream from said immobilized capture reagent, and wherein said control reagent is bound to the label component of said labeled reagent.
- 20 18. The device according to Claim 13, further comprising a cover means to enclose said strips and prevent the test sample from directly contacting said immobilized capture reagent.
- 25 19. The device according to Claim 13, wherein said handling means is a cuttable material.
20. The device according to Claim 13, wherein said handling means contains an identification means for each of the test samples and instructions for performing the assay.



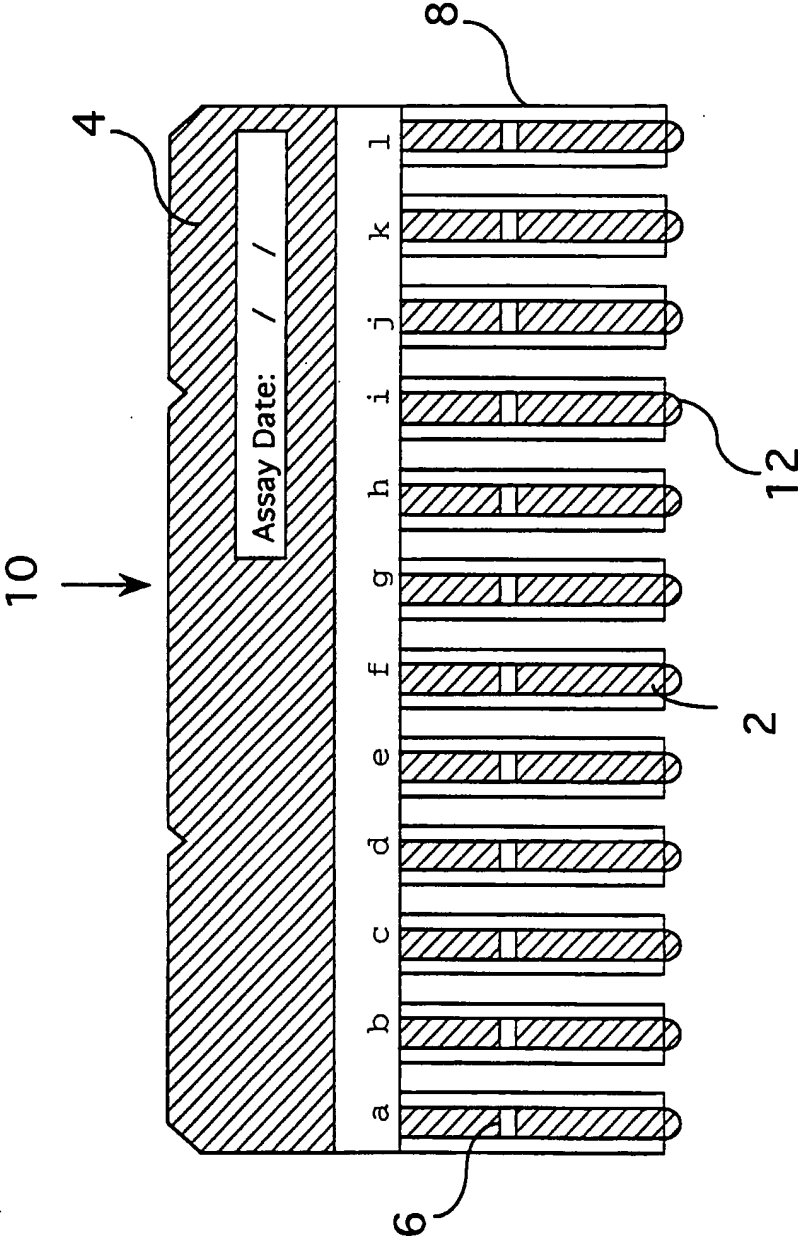


Figure 1

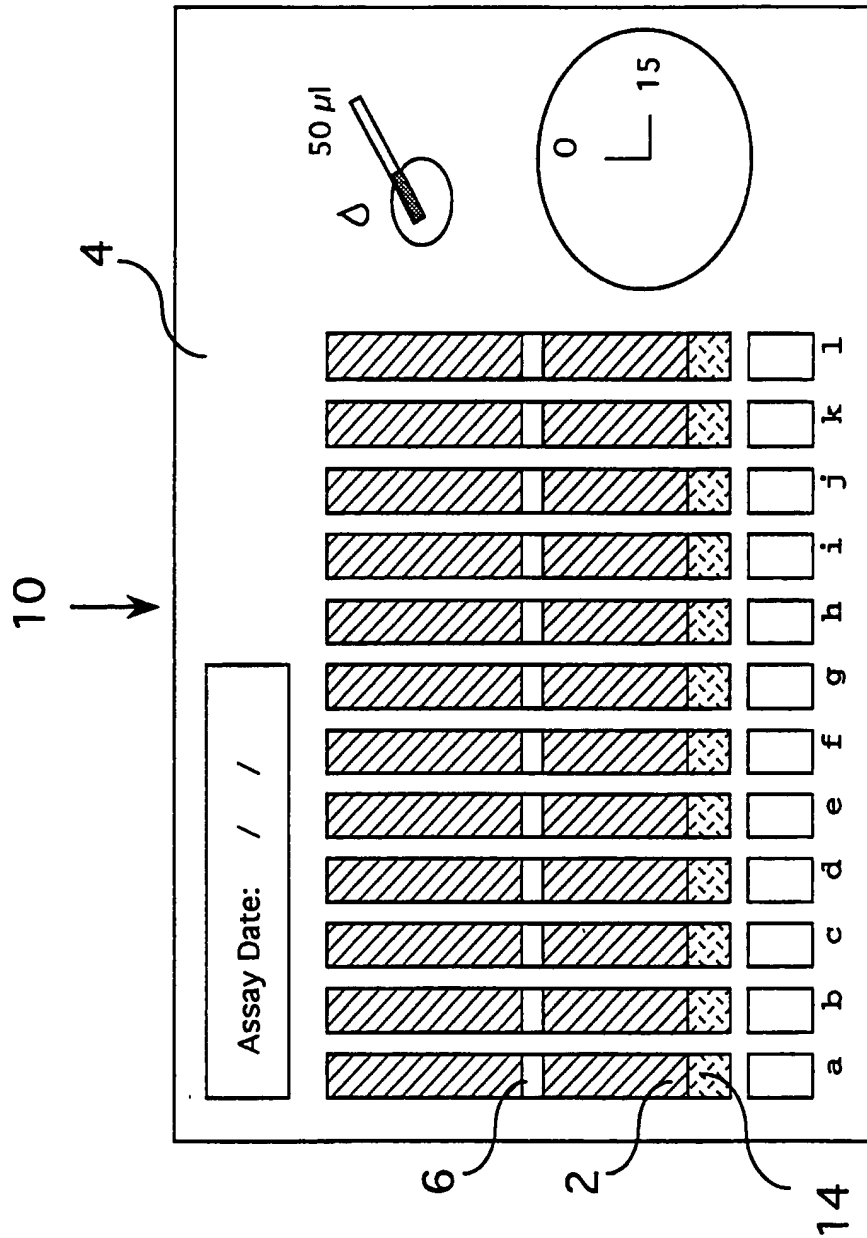


Figure 2

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/08759

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>																				
IPC(5) : C12Q 1/70; G01N 33/53, 33/538, 33/543, 33/544, 33/545																				
US CL : 422/56, 58; 435/5, 7.92; 436/518, 530, 531, 541																				
According to International Patent Classification (IPC) or to both national classification and IPC																				
<b>B. FIELDS SEARCHED</b>																				
Minimum documentation searched (classification system followed by classification symbols)																				
U.S. : 422/56, 58; 435/5, 7.92, 7.93, 7.94, 7.95, 967, 970, 973; 436/518, 530, 531, 541, 810, 820																				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched																				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)																				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
X --- Y	US, A, 4,822,565 (Köhler) 18 April 1989, col. 2, lines 43-61, claim 1, Fig. 1.	1,5 ----- 2-4,6-20																		
X --- Y	US, A, 5006,474 (Horstman et al) 09 April 1991, col. 3, line 38 - col. 7, line 4, Fig. 6.	1, 11, 12 ----- 2-10, 13-20																		
Y	EP, A, 0 323 605 (Devereaux et al) 12 July 1989, see entire document	1-20																		
Y	US, A, 5,126,276 (Fish et al) 30 June 1992, col. 2, lines 41-50, col. 3, lines 7-27, col. 4, lines 44-54.	1-20																		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																				
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>* T</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>* A* document defining the general state of the art which is not considered to be part of particular relevance</td> <td>* X</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>* E* earlier document published on or after the international filing date</td> <td>* Y</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>* G</td> <td>document member of the same patent family</td> </tr> <tr> <td>* O* document referring to an oral disclosure, use, exhibition or other means</td> <td></td> <td></td> </tr> <tr> <td>* P* document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	* A* document defining the general state of the art which is not considered to be part of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	* E* earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* G	document member of the same patent family	* O* document referring to an oral disclosure, use, exhibition or other means			* P* document published prior to the international filing date but later than the priority date claimed		
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* A* document defining the general state of the art which is not considered to be part of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																		
* E* earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																		
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* G	document member of the same patent family																		
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* P* document published prior to the international filing date but later than the priority date claimed																				
Date of the actual completion of the international search		Date of mailing of the international search report																		
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231		Authorized officer																		
Facsimile No. NOT APPLICABLE		CAROL A. SPIEGEL <i>[Signature]</i>																		
		Telephone No. (703) 308-0196																		

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/08759

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,879,215 (Weng et al) 07 November 1989	1-20
A	US, A, 4,891,321 (Hubscher) 02 January 1990	1-20
A	US, A, 5,100,621 (Berke et al) 31 March 1992	1-20
A	US, A, 5,141,850 (Cole et al) 25 August 1992	1-20
A	EP, A, 0 087 899 (Sapatino et al) 07 September 1983	1-20